

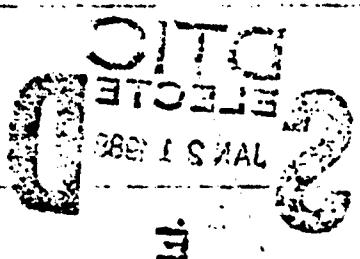
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1 PAGE		READ DIRECTIONS BEFORE COMPLETING FORM	
2. GOVT ACCESSION NO.		3. RECORDED BY CAT VALUE NUMBER	
4. FILE CODE		5. TYPE OF REPORT & PERIOD COVERED	
Acute Inhalation Toxicity of a Saline Suspension of T-2 Mycotoxin in Mice		Interim	
6. AUTHORITY		7. CONTRACT OR GRANT NUMBER	
D.A. Cressia, M.L. Nealley, L.J. Jones, III, C.G. York, R.W. Wannemacher, Jr., and D.L. Bunner		8. PROGRAM ELEMENT NUMBER, TASK AREA & WORK UNIT NUMBER	
9. PERFORMING ORGANIZATION NAME AND ADDRESS		10. REPORT DATE	
US Army Medical Research Institute of Infectious Diseases, SGMD-UIS-D Fort Detrick, Frederick, MD 21701-5011		7 November 1986	
11. CONTROLLING OFFICE NAME AND ADDRESS		12. NUMBER OF PAGES	
US Army Medical Research and Development Command		13	
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		15. SECURITY CLASS. (or Data Report)	
		16a. DECLASSIFICATION/DEGRADING SCHEDULE	
16. DISTRIBUTION STATEMENT (of this Report)		DTIC ELECTE JAN 21 1988	
Distribution unlimited - Approved for public release		S E D	
17. DISTRIBUTION STATEMENT (for the abstract entered in 8c & 30, if different from Report)		C E	
18. SUPPLEMENTARY NOTES		19. KEY WORDS (Continue on reverse side if necessary and identify by block number)	
To be published in <u>Toxicology and Applied Pharmacology</u>		Inhalation, LC ₅₀ , T-2 mycotoxin	
20. ABSTRACT (Continue on reverse side if necessary and identify by block number)		In a previous paper, we reported that inhalation of an ethanol/T-2 aerosol was 20 to 40 times more toxic to mice than systemic or dermal administration of T-2. There was concern, however, that the aerosolized ethanol vehicle might alter absorption of inhaled T-2 from the respiratory tract. In order to eliminate this question, we exposed mice to aerosols of T-2 in saline. We found that the LC ₅₀ for mice after a 10-min aerosol exposure to T-2 in saline was 0.04 ± 0.008 mg T-2/liter of air, lower than the LC ₅₀ (0.080 ± 0.02 mg T-2/liter) for an aerosol of T-2 in ethanol. We also found that, for any comparable total body	

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SECURITY CLASSIFICATION OF THIS PAGE (Block Data Enclosed)

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Acute Inhalation Toxicity of a Saline Suspension
of T-2 Mycotoxin in Mice^{1,2}

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CREASIA, D.A., NEALLEY, M.L., JONES, L.J., III, YORK, C.G., WANNEMACHER, R.W., JR., AND BUNNER, D.L. (1986). Toxicol. Appl. Pharmacol. . In a previous paper (Creasia et al., 1986), we reported that inhalation of an ethanol/T-2 aerosol was 20 to 40 times more toxic to mice than systemic or dermal administration of T-2. There was concern, however, that the aerosolized ethanol vehicle might alter absorption of inhaled T-2 from the respiratory tract. In order to eliminate this question, we exposed mice to aerosols of T-2 in saline. We found that the LC_{50} for mice after a 10-min aerosol exposure to T-2 in saline was 0.04 ± 0.008 mg T-2/liter of air, lower than the LC_{50} (0.080 ± 0.02 mg T-2/liter) for an aerosol of T-2 in ethanol. We also found that, for any comparable total body burden of T-2, mice exposed to T-2 in saline retained a greater percent of T-2 in the respiratory tract than did mice exposed to T-2 in ethanol. This suggests that the respiratory tract may be a target organ for inhaled T-2 mycotoxin. Keywords: phyto-toxins

The chemistry and systemic toxicity of T-2 and related trichothecenes in a variety of laboratory and farm animals is well documented and has been extensively reviewed. (Bomberg and Strong, 1971; Dene, 1977; Dene, 1980a, 1980b; Dene, 1981; Committee on Protection Against Trichothecene Mycotoxicosis, 1983).

The report by the Committee on Protection Against Trichothecene Mycotoxicosis (1983) noted the lack of inhalation exposure studies and recommended that the inhalation toxicity of trichothecenes be studied. Following that recommendation, this laboratory began a series of T-2 aerosol-exposure studies designed to elucidate the acute inhalation toxicity of T-2 toxin. Previous work from this laboratory (Crossie *et al.*, 1986), showed that inhalation of an aerosol of T-2 in ethanol was at least 10 times more toxic to mice than systemic administration and at least 20 times more toxic than dermal application of T-2. However, there was concern that ethanol, the T-2 aerosol vehicle, might alter the translocation of T-2 from the respiratory tract to other vital organs, which would produce systemic rather than local respiratory toxicity. Since T-2 is only slightly soluble in aqueous media, we developed the methodology for generating aerosols of T-2 toxin as a suspension in physiological saline, which eliminated the ethanol vehicle. In this paper we report the results of experiments in which mice were exposed to aerosols of T-2 in saline.

METHODS

Animals. Male Swiss ICR 5-week-old mice, were obtained from the Walter Reed Army Institute of Research (Bethesda, MD) animal breeding colony and held for 1 to 2 weeks for observation before being used in this study. All mice were held five per open-bottom polycarbonate cage, with free access to food (NIH formula 07) and water. Mice were held in rooms maintained at 75°F, 50% relative humidity, and air flow was 12 room air changes per hr. Animals used in this study had an average body weight of 30 gms.

Chemicals. Purified (>99%) T-2 mycotoxin was obtained from Mycolabs Inc., Chesterfield, Mo., as a white crystalline powder. Purity was confirmed to be >99% by both high-pressure liquid chromatography and gas chromatography-mass spectrometry analyses. Synthetic [³H]T-2 (specific activity 11 Ci/mmol; 1.1 μ mole/ml) dissolved in ethanol (ETOH) was obtained from Amersham International, Amersham U.K., for use as a tracer.

Aerosol Exposure. The inhalation chamber was designed for nose-only exposure. Basically, aerosol exposure was accomplished by placing mice in an open-ended, cone-shaped holder with only their noses protruding into a 1.5-liter aerosol chamber. The chamber was operated dynamically at 2.2 liters/min. Test aerosols were generated by atomizing cold saline suspensions of T-2 mycotoxin with a Lovelace nebulizer (Intox Products, Albuquerque, N.M.) operated at 2.2 liters/min (Mercer *et al.*, 1968). Saline suspensions of T-2 were prepared by gently grinding weighed quantities of crystalline T-2 in measured volumes of cold, physiological saline in a tissue homogenizer with a

stated clearance (0.12 to 0.15 μm) between wall and pestle (Thomas Scientific, Philadelphia, Pa). Prior to preparation of the T-2 suspension, 50 μl of [^3H]T-2 in ethanol was added to the crystalline T-2 as a tracer for aerosol mass concentration measurements, and 0.5 ml of the same solution was added as a tracer for T-2 deposition and retention studies in mice exposed to the T-2 aerosol. The ethanol solution of [^3H]T-2 was thoroughly mixed with the dry, unlabeled T-2 (50 mg) and the residual ethanol was allowed to evaporate overnight at room temperature before homogenizing the T-2 in cold saline. The mass concentration of the T-2 aerosol was varied by atomizing different concentrations of the T-2 saline suspensions and [^3H]T-2 was measured by scintillation counting (Beckman LS5800 Scintillation Counter, Beckman Instruments, Inc., Irvine, Calif.). Samples for aerosol mass concentration measurements were obtained from 1.0-liter samples taken on fiberglass filters (Gelman Scientific Inc., Ann Arbor, Mich.). The range of the aerosol mass concentrations used in this study was 1.5 to 0.002 mg T-2/liter air. Aerosol particle size was determined for each aerosol concentration with a Mercer type (Mercer et al., 1962) cascade impactor (Intox Products, Albuquerque, N.M.), operated at 100 cm^3/min . The average aerodynamic median diameter of the aerosol particles was 0.6 μm with a cg of 1.5 to 2.2. The aerosol remained as spherical liquid droplets as observed under the light microscope, indicating that the "T-2 aerosol" in this study was in actuality an aerosol of a saline suspension of T-2 and not dry T-2. All aerosol exposures were 10 min. Animals were observed at least twice daily for 2 weeks post-exposure but only those animals dying within 24 hrs post-exposure were included in the data to determine the 24 hr LC_{50} . The slope of the concentration-response lines were analyzed by probit analysis to calculate the LC_{50} 's (Finney 1977).

Retention of Inhaled T-2 Mycotoxin Aerosol. Two groups of 24 mice each were exposed to selected concentrations of T-2 aerosol. One group of 24 animals was exposed to 1.5 mg/liter and the other group of 24 animals was exposed to 0.04 mg/liter. Both groups were arbitrarily further divided after aerosol exposure into two subgroups of 12 mice each; one of which was observed for lethality for 48 hrs post-exposure while the other was killed by CO_2 asphyxiation immediately (i.e. within a few minutes) post-exposure. The intact carcasses of six of the mice killed by CO_2 asphyxiation were placed in 75 ml of 2N KOH. The intact lower respiratory tract (i.e., larynx, trachea, and lung lobes) and the nasal turbinates were removed from each of the other six mice. Lower respiratory tracts were freed from extraneous lymphoid and cardiac tissue and placed in 25 ml of 2N KOH. The external nares and surrounding skin were removed from the nasal turbinates which were also placed in 25 ml of 2N KOH. All tissues were then incubated overnight at 37°C. Triplicate 1.0-ml aliquots were subsequently taken and assayed for [^3H]T-2 (Beckman Instruments Inc., Irvine, Calif.).

RESULTS

Concentration-response data comparing lethality in mice against T-2 aerosol mass concentration are summarized in Table 1. All of the mice that died after exposure to either 1.0 or 0.5 mg/liter T-2 aerosol died within 12 hr. The single aberrant mouse that survived exposure to 0.5 mg/liter T-2, survived for the full 2-week observation period without exhibiting any obvious signs of toxicity. Mice exposed to either 0.07, 0.1, or 0.15 mg/liter T-2 aerosol all died within 24 hr. All mice that died after exposure to aerosol mass concentrations of 1.0 to 0.07 mg/liter tended to appear relatively normal

when compared immediately after aerosol exposure to control animals. By 4 to 6 hr, however, these mice became lethargic, remained separate from their cagemates, assumed a hunched body position, and died in that position. Mice exposed to <0.07 mg/liter T-2 also appeared normal immediately after aerosol exposure, but began to exhibit signs of toxicity at 4 to 6 hr post-exposure. Depending on the aerosol mass concentration, a number of these animals recovered, and some survived for the 2-week observation period. Mice that survived for 24 hrs but died within the 2 wk observation period after exposure to the lower aerosol concentrations (i.e. < 0.07 mg/liter) died at staggered, unpredictable time points, but mostly within 24 to 30 hr post exposure. We calculated the 24 hr LC₅₀ for mice of aerosolized T-2 in saline to be 0.04 ± 0.008 mg/liter.

Retention of Inhaled T-2.

In the group of 24 mice exposed to 1.5 mg T-2/liter, 12 out of the 12 mice arbitrarily selected for 48 hr observation died within 10 hr post aerosol. These data are summarized in Table 2. Scintillation counts of [³H]T-2 in tissues of mice in the other subgroup of 12 mice showed that 32 ± 3 µg equivalents of T-2 were retained immediately after a 10-min exposure. Of that, 12.5% was retained in the nasal turbinates and 5.6% in the respiratory tract. The remaining [³H] (81.9%) was distributed throughout the rest of the carcass.

From the group of 24 mice exposed to 0.04 mg T-2/liter air, (LC₅₀) six of the 12 mice arbitrarily selected for 48-hr observation died within 15-hr post exposure. Scintillation counts of [³H]T-2 in tissues of mice in the other

subgroup of 12 mice showed that 17 ± 1.4 μg equivalents of T-2 were retained immediately after a 10-min exposure. Of this total, 22.2% of T-2 was retained in the nasal turbinates and 2.4% retained in the respiratory tract. The remaining T-2 (75.4%) was distributed throughout the rest of the carcass.

DISCUSSIONS

The acute inhalation toxicity of aerosols of T-2 in saline was investigated. We found that the 24 hr LC_{50} for mice exposed for 10 min to this aerosol was 0.041 ± 0.008 mg/liter . When we measured total body retention of μg equivalents of T-2 in mice exposed for 10 min to either 1.5 or 0.04 mg/liter T-2, we found a total body burden of 32 μg and 17 μg , respectively. The latter aerosol mass concentration (i.e. 0.04 mg/liter) was an LC_{50} , and the total T-2 retained (i.e., 17 μg) was equivalent to an LD_{50} of 0.6 mg/kg body weight.

The above data is quantitatively different from those previously reported from this laboratory (Creasia *et al.*, 1986) for a study in which mice were exposed under similar conditions to aerosols of T-2 in ethanol. In the previous study we found the LC_{50} for T-2 in ethanol to be 0.24 mg/liter for mice of a similar age and weight range as those used in this study. More importantly, when we (Creasia *et al.*, 1986) previously measured the total body burden of T-2 (expressed as μg equivalents of T-2) in mice exposed to a T-2/ethanol aerosol of 0.24 mg/liter (i.e. the ethanol-T-2 LC_{50} ; 6 of 12 mice so exposed died within 24 hrs.) we found the total body burden of T-2 to be 28 ± 1.5 μg T-2. Data presented in this report, however, show that a total body burden of 32 ± 3 μg T-2 was sufficient to kill 100% of mice exposed to T-2 in saline. If the lethality observed in mice after inhalation of T-2 mycotoxin was a

result of systemic toxicity of T-2 (i.e. total body burden), the death rates of mice exposed to aerosols of either a saline suspension or an ethanol solution of T-2 should be similar when the total body burden is similar, (i.e. 28 μ g and 32 μ g), regardless of the type of T-2 aerosol. Since this is obviously not the case, we postulate that acute toxicity from inhaled T-2 is manifested through organ burden (i.e. target organ), rather than total body burden, and that the respiratory tract is a likely target organ. Studies are currently underway in this laboratory to investigate this theory.

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In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

ACKNOWLEDGEMENTS

The authors thank Dr. E. H. Stephenson, and Mr. W. C. Patrick, III, for review of this manuscript; Ms. K. F. Kenyon and Mr. E. C. Hauer for editorial service, and Ms. P. L. Weicht for typing this manuscript.

TABLE 1

ACUTE INHALATION TOXICITY OF A SALINE SUSPENSION OF
T-2 MYCOTOXIN IN MICE^a

Aerosol Mass Concentration (mg/Liter)	Dead/Exposed (24 hrs)
1.0	12/12
0.5	11/12
0.15	12/12
0.1	12/12
0.07	10/12
0.04	7/12
0.03	2/12
0.002	1/12
Saline Control	0/12

$LC_{50} = 0.041 \pm 0.008$ mg/liter

^a 10-Min aerosol exposure

TABLE 2

RETENTION OF INHALED T-2 MICOTOXIN IN MICE EXPOSED TO
AN AEROSOL OF A SALINE SUSPENSION OF T-2^a

Aerosol Mass Concentration (mg/liter)	Dead/Exposed	X Distribution		
		Lower	Respiratory	Nasal
1.5	12/12	32 ± 3	5.6 ± 0.4	12.5 ± 6.2
0.04	6/12	17 ± 1.4	2.4 ± 0.2	22.2 ± 3.4

^a 10-Minute aerosol exposure

^b N = 6; mean ± SD

^c Remains after lower respiratory tract and nasal turbinates removed

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Date of Clearance: 28 October 1986

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